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Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold

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Abstract

Germination of *Botrytis cinerea* spores on potato dextrose agar after a 30 s immersion in 10 or 20% ethanol was 87 and 56%, respectively, compared to 99% among untreated controls. After similar immersion in 0.5 or 1.0% potassium sorbate, 84 and 68% of the spores germinated, respectively. Addition of 0.5 and 1.0% potassium sorbate to 10 and 20% ethanol solution significantly increased the inhibition of spore germination. The germination of spores after 30 s immersion in 20% ethanol plus 0.5% potassium sorbate was 9.7%. The incidence of gray mold, caused by *B. cinerea*, on detached berries of 'Flame Seedless' grapes immersed for 30 s in water, 10 and 20% ethanol, and 0.5 or 1.0% potassium sorbate was 55.2, 42.1, 31.0, 37.7, or 24.4%, respectively. Addition of 0.5 and 1.0% potassium sorbate to 10 and 20% ethanol reduced decay to 10% or less and was more effective than either alone. After 30 days of storage at 1 °C, the combination of 20% ethanol either with 0.5 or 1.0% potassium sorbate was equal in efficacy to commercial SO_2 generator pads in reducing the incidence of gray mold on 'Thompson Seedless' grapes. None of the combinations of ethanol and potassium sorbate injured the berries.

Keywords: Grey mold; *Vitis vinifera*; Alcohol

1. Introduction

Botrytis cinerea Pers. is the most common postharvest pathogen of table grapes in most regions of the world (Bulit and Dubos, 1988). Currently, postharvest diseases of table grapes are controlled by the

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postharvest application of SO_2, either by weekly fumigation in storage rooms or by packing grapes in polyethylene-lined boxes with SO_2 generator pads. Problems associated with SO_2 use include the following: (1) SO_2 residues that exceed the tolerance of 10 mg/kg of most countries, which can occur if the gas dosage is too high; (2) unsightly bleaching injuries that can occur to berries after numerous or high dosage fumigations; (3) SO_2 cannot be used on organically certified grapes (Gabler and Smilanick, 2001); and (4) because of sulfite hypersensitivity in some people, the dietary hazard of SO_2 was recognized and it was removed from the US Food and Drug Administration 'generally regarded as safe' classification in 1986 (Zahavi et al., 2000). Therefore, the development of alternative strategies to control postharvest decay of table grapes that are safe, effective, economical, and compatible with commercial handling is of interest.

Ethanol and potassium sorbate are common food additives with potent antimicrobial activity (Sofos, 1989; Larson and Morton, 1991). Ethanol dips and vapors have been reported to control postharvest diseases of peaches, citrus fruit, and table grapes (Larson and Morton, 1991; Feliciano et al., 1992; Smilanick et al., 1995; Gabler and Smilanick, 2001; Gabler et al., 2002; Karabulut et al., 2003), especially when heated (Smilanick et al., 1995; Margosan et al., 1997; Karabulut et al., 2004). Sorbates are common food preservatives for many applications, and its spectrum of activity includes *B. cinerea* (Sofos, 1989), although there are no reports where it was evaluated to control postharvest decay of table grapes. Potassium sorbate applied after harvest controlled a variety of postharvest pathogens on citrus (Wild, 1987; Palou et al., 2002), and sweet cherry (Karabulut et al., 2001; Mari et al., 2004).

Previous studies demonstrated a gradual increase in the sporocidal activity of ethanol with an increase in ethanol concentration. Concentrations greater than 30% killed spores of *B. cinerea* rapidly, while those 20% and below were sublethal (Lichter et al., 2002; Karabulut et al., 2003, 2004). The use of higher concentrations of ethanol concentration incurs additional ethanol costs and exacerbates safety hazards and disposal issues that can reduce the feasibility of ethanol use. The flammability limit of ethanol is 33 ml/l and the air in manned workplaces cannot contain more than 1 ml/l (Gabler et al., 2005). In addition, commercial SO_2 generator sheets are superior to ethanol in effectiveness for the control of postharvest decay, even when the ethanol was used at high and sporocidal levels. Ethanol efficacy declines during prolonged storage, because its residues are low and short-lived, suggesting that deep secondary *B. cinerea* infections during storage are not controlled. These issues are disadvantages of postharvest ethanol applications, and can limit the use of ethanol in practice.

Therefore, the objective of this study was to evaluate the efficacy of the sublethal concentrations of ethanol, in combination with potassium sorbate, to control gray mold on table grapes.

2. Materials and methods

2.1. Fruit

Mid-season organically grown 'Flame Seedless' and 'Thompson Seedless' grapes (*Vitis vinifera*) were commercially harvested from vineyards located in the San Joaquin Valley of California. The grapes were used on the day of harvest.

2.2. Fungi

B. cinerea was isolated from infected grape berries and cultured on potato dextrose agar (PDA: Difco, Detroit, USA). Spores were harvested from 2-week-old PDA cultures of *B. cinerea* grown at 25 °C. An amount of 5 ml of sterile water, containing 0.05% (v/v) Triton X-100, was added to a petri plate culture, the spores were gently dislodged from the surface with a sterile glass rod, and suspensions were filtered through three layers of cheesecloth to remove mycelial fragments. The suspensions were diluted with sterile water to an absorbance of 0.25 at 425 nm as determined by a spectrophotometer. This density contained 1.2×10^6 conidia/ml. Further dilutions with sterile water were made to obtain the desired spore concentrations.

2.3. Ethanol and potassium sorbate toxicity to B. cinerea spores

B. cinerea spores (10,000 spores/ml) were mixed with various ethanol concentrations, either alone or in combination with 0.5 or 1.0% potassium sorbate at

ambient temperature (22–24 °C) in a final volume of 2 ml. The pH of 0.5 and 1.0% potassium sorbate solutions was 9.1 and 9.3, respectively. Addition of 10 and 20% ethanol either to 0.5 or 1.0% potassium sorbate did not change the initial pH of solutions. After 30 s, the spore suspensions were diluted 100-fold in sterile water and 100 μl were plated on PDA. After 48 h incubation at 24 °C, the colonies per plate were counted. Data were expressed as the percentage of germinated spores. The experiment was performed twice.

2.4. Ethanol and potassium sorbate treatment of table grapes

Two types of experiments were performed to evaluate the efficacy of sublethal levels of ethanol alone or in combination with potassium sorbate to control gray mold of table grape. In the first type of experiment, berries were cut from the rachis with pedicel intact. The berries were then inoculated with a *B. cinerea* (10^5 conidia/ml) spore suspension. A volume of 50 ml of inoculum was sprayed on about 900 berries, which were then dried in air for 30 min and then immersed for 30 s in 10 and 20% (v/v) ethanol solutions alone or in combination with 0.5 and 1.0% (w/v) potassium sorbate. A volume of 1 l of each solution was used to immerse 90 berries. After treatment, the single berries were dried in air for 30 min and then placed on metal racks in covered plastic boxes lined with moist paper towels. The number of berries with gray mold was counted after 10 days of incubation at 15 °C. The experiment was performed twice. Each replicate consisted of 30 single berries and three replicates were used for each treatment.

In the second series of experiments, entire clusters of grapes were harvested, treated, dried, and stored in ventilated polyethylene (VPE) bags containing about 800–1000 g of grapes each. The clusters were placed on metal racks as a single layer (out of VPE bags) and inoculated as described previously. A volume of 100 ml of inoculum was sprayed on to 40 kg clusters. Inoculated clusters were immersed for 30 s in 10 and 20% (v/v) ethanol solutions alone or in combination with 0.5 and 1.0% (w/v) potassium sorbate. The fruit were immersed in the solutions while contained within the VPE bags. A volume of 5 l of each solution was used to immerse 4 kg fruit. After treatment, the fruit were removed from the bags, air dried for about 1 h, and placed into new VPE bags, placed in fiberboard boxes, stored at 0–1 °C (RH > 90%) for 24 h to facilitate rapid cooling, then wrapped with polyethylene stretch film (20 μm) to minimize weight loss and stem desiccation and stored for 30 days at 0–1 °C (RH > 90%). SO_2 treatment consisted of dual-release generator pads containing 7 g of sodium metabisulfite (Uvas, Santiago, Chile). Two generator pads were placed on top and two beneath the grapes within each box and then, boxes were enclosed within plastic bags just prior to storage. The number of decayed berries per kg of fruit was recorded after storage. Each replicate consisted four VPE bags containing about 900 g of grapes each. The experiment was performed twice.

2.5. Statistical analysis

An analysis of variance was applied to the results of each experiment. Incidence data and number of decayed berries per kg of fruit were transformed (arcsin of the square root of the proportion of affected fruit) before analysis. Means were separated using Fisher's LSD ($P \leq 0.05$). We apply the term synergy as defined by Richer (1987), where the effectiveness of a combination of treatments exceeds the prediction of the effectiveness of their additive action estimated by Limpel's formula ($E_e = X + Y - (XY/100)$).

3. Results and discussion

3.1. Spore mortality

Exposure of 30 s of *B. cinerea* spores to 10 or 20% ethanol reduced germination to 87 and 56%, respectively. Spores similarly immersed in 0.5 or 1.0% potassium sorbate later germinated at 84 and 68%, respectively. Addition of 0.5 and 1.0% potassium sorbate to 10 and 20% ethanol solution significantly increased the inhibition of spore germination. The highest efficacy in inhibiting the spore germination was achieved by the combination of 20% ethanol with 0.5 and 1.0% potassium sorbate (Fig. 1). In previous studies, the germination of spores of *B. cinerea* immersed for 10 s in 10 and 20% ethanol at ambient temperatures was reduced, while immersion in 30–40% ethanol completely inhibited spore germination (Lichter et al., 2002; Karabulut et al., 2004). In these studies, brief

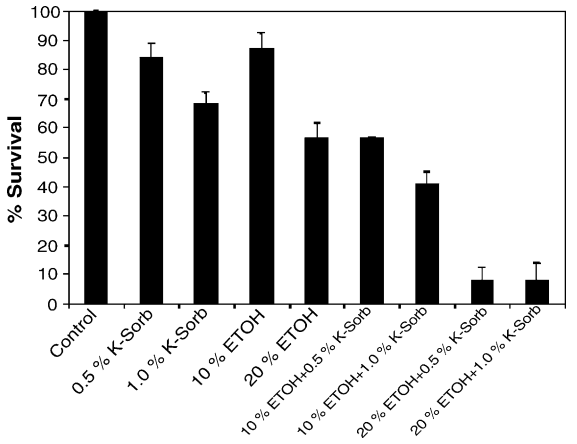

Fig. 1. Germination on potato dextrose agar of *B. cinerea* spores after immersion in 10 or 20% ethanol alone or in combination with 0.5 or 1.0% potassium sorbate (K-Sorb).

exposures to ethanol at concentrations of 20% or less were not lethal to *B. cinerea*. Our results indicate that brief exposure of spores to 0.5 and 1.0% potassium sorbate reduced germination of only a portion of the spores treated. Sofos (1989) reviewed the subject of antifungal activity of sorbates, and reported a constant concentration of 0.05–0.15% was needed to inhibit the growth of many fungi in foods, and that concentration was influenced by the pH and temperature. Lopez-Malo et al. (2002) indicated that potassium sorbate inhibited the in vitro growth of *Aspergillus flavus*.

3.2. Decay control

In the first experiment, the decay incidence of detached berries of 'Flame Seedless' grapes immersed in water, 10 and 20% ethanol, 0.5 and 1.0% potassium sorbate, was 55.2, 42.1, 31.0, 37.7, 24.4%, respectively. Addition of 0.5 and 1.0% potassium sorbate to 10 and 20% ethanol caused a synergistic improvement in these treatments (Fig. 2A). The results of the second experiment conducted on detached berries of 'Thompson Seedless' grapes supported the results of the previous experiment. The efficacy of the combinations of 10 and 20% ethanol with 0.5 and 1.0% potassium sorbate was equal and significantly superior to the stand-alone applications of both treatments (Fig. 2B).

In the first storage experiment conducted on clusters of 'Thompson Seedless' grapes, the efficacy of the combinations containing 10% ethanol and 1.0% potassium sorbate, 20% ethanol and either 0.5 or 1.0% potassium sorbate was equal to that of SO_2 (Fig. 3A). In another repeated storage experiment, the combination of 20% ethanol either with 0.5 or 1.0% potassium

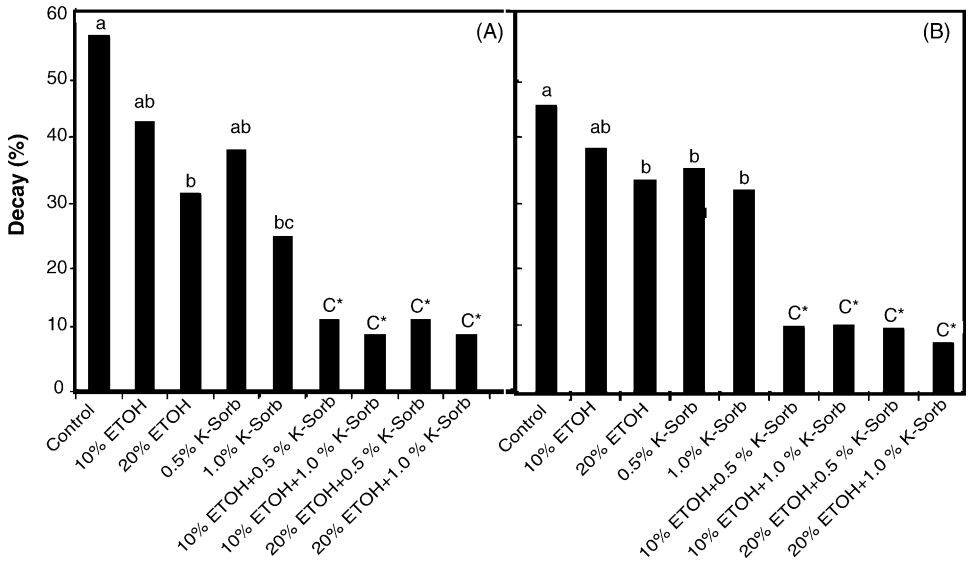

Fig. 2. Incidence of gray mold on detached 'Flame Seedless' (A) and 'Thompson Seedless' (B) berries inoculated with spores of *B. cinerea* prior to immersion for 30 s in 10 or 20% ethanol alone or in combination with 0.5 or 1.0% potassium sorbate (K-Sorb) and storage for 10 days at 15 °C. Columns with unlike letters differ significantly ($P \leq 0.05$). (*) Synergistic effect according to Limpel's formula.

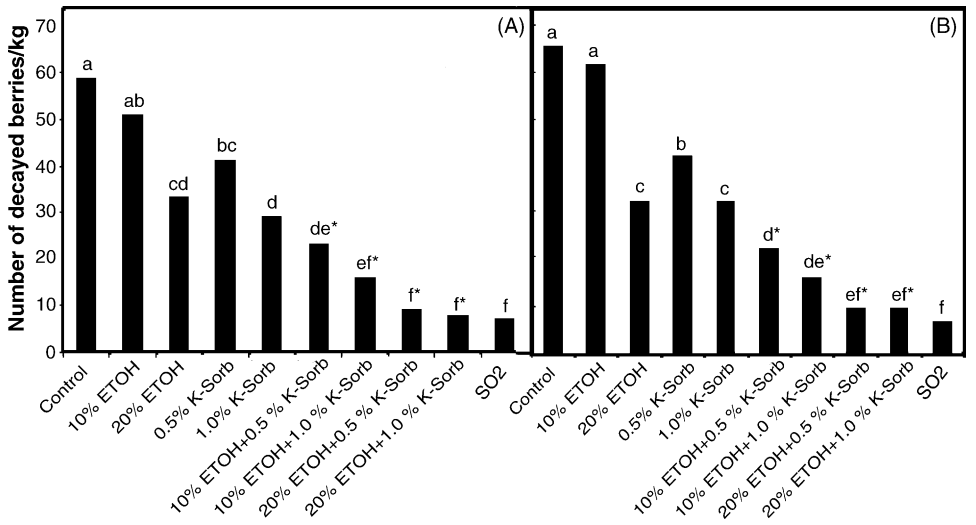

Fig. 3. Incidence of gray mold on 'Thompson Seedless' clusters inoculated with spores of *B. cinerea* prior to immersion for 30 s in 10 or 20% ethanol alone or in combination with 0.5 or 1.0% potassium sorbate (K-Sorb) and storage for 30 days at 1 °C. The experiment was performed twice, (A) and (B) represent the first and second experiment, respectively. Columns with unlike letters differ significantly ($P \leq 0.05$). (*) Synergistic effect according to Limpel's formula.

sorbate was equal to SO_2 in the efficacy (Fig. 3B). None of the combinations of ethanol with potassium sorbate caused a surface injury to the berries or altered the appearance of the raches of clusters. The efficacy of 20% ethanol as a stand-alone treatment was about 50% and this finding is in agreement with the results of a previous study (Lichter et al., 2002). Ethanol treatment efficacy declined after prolonged storage indicating the ineffectiveness of ethanol against secondary *Botrytis* infections during storage (Lichter et al., 2002). This issue is the biggest disadvantage of postharvest ethanol applications that can limit the use of ethanol in practice. Ethanol is a volatile compound and quickly evaporates from the berry surface during storage, therefore, it is ineffective against secondary infections (Gabler et al., 2005). In addition to increased efficacy of ethanol on spore mortality when used in combination with potassium sorbate, the existence of potassium sorbate during storage may protect the berries from secondary infections that usually occur through the end of storage. Potassium sorbate applied after harvest controlled a variety of postharvest pathogens in citrus (Wild, 1987; Palou et al., 2002), and sweet cherry (Karabulut et al., 2001; Mari et al., 2004).

In conclusion, our results indicate that the use of sublethal levels of ethanol in combination with potassium sorbate could be effective in controlling postharvest diseases of grapes caused by *B. cinerea*. Regulatory issues associated with the approval of these compounds should be minimal.

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